Am. J. Trop. Med. Hyg., 83(6), 2010, pp. 1245–1248
doi:10.4269/ajtmh.2010.10-0279

Seroprevalence Survey of Chikungunya Virus in Bagan Panchor, Malaysia

Said Mas Ayu, Li Rong Lai, Yoke Fun Chan, Ahmad Hatim, Noran Naqiah Hairi, Asiah Ayob, and I-Ching Sam*

Department of Social and Preventive Medicine, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia; Tropical Infectious Diseases Research and Education Centre, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia; Department of Psychological Medicine, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia; Communicable Diseases Control Unit, State Health Department, Perak, Malaysia

Abstract. In 2006, an outbreak of Chikungunya virus (CHIKV) of the Asian genotype affected over 200 people in Bagan Panchor village in Malaysia. One year later, a post-outbreak survey was performed to determine attack rate, asymptomatic rate, and post-infection sequelae. Findings were compared with recent CHIKV outbreaks of the Central/East African genotype. A total of 180 residents were interviewed for acute symptoms and post-infection physical quality of life and depressive symptoms. Sera from 72 residents were tested for CHIKV neutralizing antibodies. The estimated attack rate was 55.6%, and 17.5% of infected residents were asymptomatic. Arthralgia was reported up to 3 months after infection, but there were no reports of long-term functional dependence or depression. Symptomatic and seropositive residents were significantly more likely to live in the area with the most dense housing and commercial activities. CHIKV had a high attack rate and considerable clinical impact during the Bagan Panchor outbreak.

INTRODUCTION

Chikungunya virus (CHIKV), an alphavirus transmitted by *Aedes* mosquitoes, causes epidemic fever, rash, and arthralgia. CHIKV is endemic in Africa and Asia, where numerous outbreaks have been reported since the 1950s. There are three genotypes of CHIKV, which are the West African, Central/East African (CEA), and Asian genotypes. Since 2005, CHIKV of the CEA genotype has spread worldwide from initial outbreaks on Indian Ocean islands and in India and has affected millions.

The *Aedes* vectors for CHIKV are endemic in Malaysia. However, the first confirmed outbreak of CHIKV only occurred recently and was confined only to Klang in 1998.[1] A second limited outbreak occurred in Bagan Panchor, about 200 km northwest of Klang, in 2006,[2,3] representing a reappearance of CHIKV in Malaysia after an 8-year hiatus. This pattern of reemergence after several years has been described in other countries.[4] The Bagan Panchor outbreak was caused by the endemic Asian genotype, similar to that identified in the 1998 outbreak in Klang but distinct from the CEA genotype that was causing the worldwide epidemic at the time. The epidemic CEA genotype was subsequently imported into Malaysia, leading to another small outbreak in Ipoh (2006),[5] before causing the nationwide outbreak that has been ongoing since 2008.[6]

Seroprevalence surveys after outbreaks of Asian CHIKV are scarce. They are potentially of interest, because they may provide insight as to why the Asian genotype has never caused epidemics on a global scale as the recent CEA strains have. In 2007, 1 year after the outbreak, we visited Bagan Panchor to determine seroprevalence, asymptomatic infection rates, and sequelae of CHIKV infection. We compared our findings with studies of the recent epidemic CEA genotype.

MATERIALS AND METHODS

Bagan Panchor (4°31′N, 100°37′E) is a small fishing village in the state of Perak, located on the northwest coast of Malaysia, with a predominantly Chinese population of approximately 1,500 (Figure 1).[7] The study was conducted in March 2007, about 1 year after the CHIKV outbreak, which lasted from January to April 2006. During the outbreak, the Ministry of Health identified over 240 suspected symptomatic cases with at least one of the three main CHIKV symptoms of fever, rash, or arthralgia. Approval was obtained from the Medical Ethics Committee of the University Malaya Medical Center. Support for the study was obtained from community leaders of Bagan Panchor.

The investigating team conducted house-to-house interviews by visiting all houses in the village using a structured questionnaire in Mandarin. The location of the houses was described as being in the southeastern (area A) or northwestern (area B) half of the village (Figure 1). All those who took part gave informed consent. For children unable to answer the questions, their parents or legal guardians were consented and interviewed. Participants were first asked demographic questions and then asked if they had experienced acute symptoms consistent with CHIKV infection during the outbreak period of January to April 2006. Symptomatic participants were defined as those with at least one of the three main CHIKV symptoms of fever, rash, or arthralgia. Those who were symptomatic were further subjected to a Barthel Index (BI) questionnaire and a Mini International Neuropsychiatric Interview (MINI) to determine symptoms of functional dependence and depression up until the time of the study in March 2007. The BI is a scale measuring ability to carry out certain activities of daily living in self-care (e.g., feeding and bathing) and mobility (e.g., ambulation and climbing stairs), with a maximum score of 100 indicating physical independence.[8] The MINI is a screening tool for identifying symptoms of a major depressive episode.[9]

To determine seropositivity to CHIKV, neutralization was carried out for each serum sample in 96-well microplates (BD Biosciences, San Jose, CA). The microplates were seeded with 10^4 Vero cells and incubated overnight at 37°C with 5% CO_2. Test serum was 2-fold serially diluted with serum-free media. A positive control (rabbit anti-CHIKV serum) and negative controls (serum from an uninfected person and serum-free media) were included in each plate. A CHIKV strain (MY/0306/37348) isolated from a Bagan Panchor patient was used as the viral antigen at a 50% tissue culture infectious dose of 10^3/mL. Serum was heat-inactivated at 56°C for 30 minutes to inactivate complement. CHIKV was incubated

* Address correspondence to I-Ching Sam, Tropical Infectious Diseases Research and Education Centre, Faculty of Medicine, University Malaya, Kuala Lumpur 50603, Malaysia. E-mail: jicsam@um.edu.my

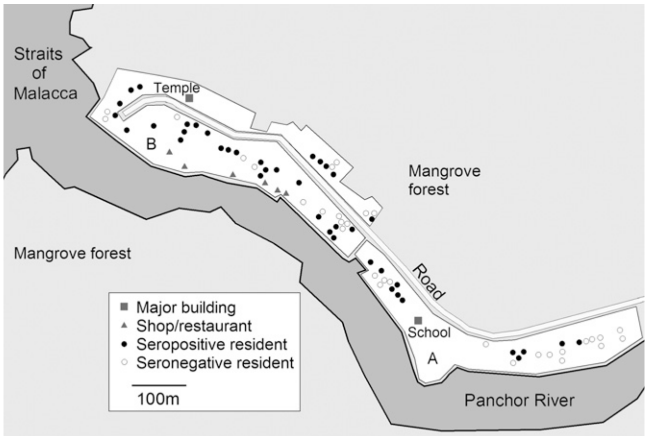

FIGURE 1. Representation of Bagan Panchor village, which is arranged along a 1-km road running from southeast to northwest adjacent to the Panchor River as it flows into the Straits of Malacca. The village is surrounded by a mangrove forest. The residences of seropositive and seronegative residents within areas A and B are shown. Seropositive cases were more likely to reside within area B, which is also where the shops and restaurants are located.

with an equal volume of serum at 37°C for 1 hour. This antigen–serum mixture was then added to the Vero cells in the microplate and shaken for 1 hour. Excess suspension was removed, cells were briefly washed with serum-free media, and Eagle's Minimum Essential Medium with 2% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA) was added for maintenance. The cells in the microplate were observed for characteristic cytopathic effect. The neutralization titer for a given serum sample was determined by the highest dilution at which that serum neutralized the virus at day 7. Serum that contained no neutralizing activity did not inhibit cytopathic effects and was considered to be seronegative. Serum with neutralizing activity was considered to be seropositive, indicating laboratory-confirmed past CHIKV infection. Each serum sample was tested in triplicate.

Data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL). Descriptive analysis was performed on demographic and symptoms data. For univariate analysis, Mann-Whitney U tests were used for continuous variables, and χ^2 or Fisher's exact tests were used for categorical variables. Logistic regression analyses were also carried out to determine demographic predictors of two separate outcomes: symptomatic disease and CHIKV seropositivity. Univariate logistic regression was initially carried out for each variable. Those significant at $P < 0.2$ were entered into the multivariate regression model based on the likelihood ratio test. Final P values of < 0.05 were considered as significant.

RESULTS

There were about 150 houses in total in the village. A total of 180 residents, or 12% of the estimated 1,500 people, from 80 houses agreed to be interviewed (Table 1) (mean age = 38.2 years; range = 1–77 years). The 80 houses where the interviewed participants lived were spread throughout the village; there was no difference between the percentages of houses sampled in area A and area B (47.9% versus 58.2%; $\chi^2 = 1.6$, $P = 0.21$). Of the 180 residents interviewed, 72 (40.0%) agreed to have their serum tested (mean age = 44.0 years; range = 12–77 years). Of the 72 people tested, 40 (55.6%) were seropositive, and 7 (17.5%) of these were asymptomatic. Of the tested acutely symptomatic participants, 33 of 38 (86.8%) were seropositive; 7 of 34 (20.6%) tested asymptomatic participants were seropositive. Three of seven asymptomatic seropositive residents had a symptomatic household member.

TABLE 1
Demographic characteristics of study participants

	Participants interviewed				Participants tested for CHIKV			
	Total (N = 180)	Asymptomatic (N = 109)	Symptomatic (N = 71)	Univariate analysis (P value)	Total (N = 72)	Seronegative (N = 32)	Seropositive (N = 40)	Univariate analysis (P value)
Age (years)								
≤ 18 years	38	26 (68.4%)	12 (31.6%)	0.15	5	3 (60.0%)	2 (40.0%)	0.32
19–49 years	79	49 (62.0%)	30 (38.0%)	0.33	39	19 (48.7%)	20 (51.3%)	0.29
≥ 50 years	63	34 (54.0%)	29 (46.0%)	Reference	28	10 (35.7%)	18 (64.3%)	Reference
Sex								
Male	84	53 (63.1%)	31 (36.9%)	Reference	38	20 (52.6%)	18 (47.4%)	Reference
Female	96	56 (58.3%)	40 (41.7%)	0.52	34	12 (35.3%)	22 (64.7%)	0.14
Residential area								
A	82	62 (75.6%)	20 (24.4%)	Reference	28	17 (60.7%)	11 (39.3%)	Reference
B	98	47 (48.0%)	51 (52.0%)	< 0.001*	44	15 (34.1%)	29 (65.9%)	0.03†
Education‡								
Primary	77	43 (55.8%)	34 (44.2%)	Reference	39	13 (33.3%)	26 (66.7%)	Reference
Secondary or tertiary	41	26 (63.4%)	15 (36.6%)	0.43	17	9 (52.9%)	8 (47.1%)	0.17
Child ≤ 18 years	38	27 (71.1%)	11 (28.9%)	0.12	5	4 (80.0%)	1 (20.0%)	0.08
No formal education	23	12 (52.2%)	11 (47.8%)	0.76	10	5 (50.0%)	5 (50.0%)	0.33
Occupation‡								
Housewife	56	31 (55.4%)	25 (44.6%)	Reference	22	8 (36.4%)	14 (63.6%)	Reference
Student	38	27 (71.1%)	11 (28.9%)	0.13	5	4 (80.0%)	1 (20.0%)	0.11
Fisherman	24	13 (54.2%)	11 (45.8%)	0.92	16	7 (43.8%)	9 (56.3%)	0.65
Unemployed	14	10 (71.4%)	4 (28.6%)	0.28	8	3 (37.5%)	5 (62.5%)	0.95
Businessman	12	5 (41.7%)	7 (58.3%)	0.70	4	1 (25.0%)	3 (75.0%)	0.44
Retired	10	7 (70.0%)	3 (30.0%)	0.39	4	3 (75.0%)	1 (25.0%)	0.66
Other	25	15 (60.0%)	10 (40.0%)	0.39	12	6 (50.0%)	6 (50.0%)	0.18

*Living in area B was retained in the final regression model as a predictor of being symptomatic (adjusted odds ratio = 3.4; 95% confidence intervals = 1.8–6.4; $P < 0.001$).
†Living in area B was retained in the final regression model as a predictor of being seropositive for CHIKV (adjusted odds ratio = 3.0; 95% confidence intervals = 1.1–7.9; $P = 0.029$).
‡Data is missing for one patient.

TABLE 2
Clinical features of seropositive residents with laboratory-confirmed Chikungunya infection ($N = 40$)

	Number/total	%
Asymptomatic	7/40	17.5
Symptomatic	33/40	82.5
Arthralgia	30/33	90.9
Knees	22/30	73.3
Ankles	20/30	66.7
Elbows	20/30	66.7
Wrists	15/30	50.0
Fingers	12/30	40.0
Shoulders	6/30	20.0
Spine	2/30	6.7
Fever	25/33	75.8
Rash	21/33	63.6
Myalgia	16/33	48.5
Headache	10/33	30.3
Pruritus	8/33	24.2
Nausea	1/33	3.0
Bleeding	0/33	0

Symptomatic and seropositive cases were reported throughout the village, although there were more cases in the northwestern half by the mouth of the river (area B in Figure 1), which had more dense housing, the jetty, shops, and restaurants. This was confirmed by multivariate analyses; the only significant independent demographic predictor of both symptomatic disease and seropositivity was residence in area B (Table 1). Other demographics such as age, sex, education background, and occupation were not significant predictors.

The clinical features of the 40 seropositive residents are shown in Table 2. Of the 33 symptomatic cases, the most common symptoms were arthralgia (90.9%), fever (75.8%), and rash (63.6%). The most frequently affected joints were knees, ankles, elbows, and wrists. Of the 30 seropositive cases reporting arthralgia, 29 stated that joint symptoms lasted for a median of 14 days (range = 2–100 days).

Two (5.0%) seropositive people reported dependence on others for self-care activities such as using the toilet and dressing, and one of two people required a wheelchair. This dependence was temporary, lasting between 3 and 30 days. Only one of the seropositive residents, who had arthralgia symptoms for 100 days, reported concurrent depressive symptoms of loss of interest and tiredness; this was insufficient to meet the criteria for a major depressive episode.

DISCUSSION

This study is the first seroprevalence study to be performed in Malaysia since the 1960s, when "a very low level of population immunity" was found in the western states, including Perak.[10] A total of 72 Bagan Panchor residents were tested, which is higher than the 63 tested during the outbreak.[2,3]

Compared with the contemporary Indian Ocean outbreaks, the attack rate in Bagan Panchor of 55.6% was higher than the rates in Mayotte[11] and Réunion Island[12] but lower than the rates in the Comoros[13] and Lamu Island in Kenya[14] (Table 3). The Bagan Panchor rate may overestimate the true attack rate, because symptomatic participants were more likely to agree to testing than the asymptomatic participants (53.5% versus 31.2%; $\chi^2 = 8.9$, $P = 0.003$). Differences in attack rates are likely caused by local factors, including population immunity and genetic susceptibility, vector competence, environmental settings, and effectiveness of outbreak control measures. These may also vary within the same locality. We found that residence in the northwestern half of the village, where the main commercial activities and a greater density of housing were located, predicted symptomatic disease and seropositivity. We did not explicitly study the reasons for this in Bagan Panchor, but it is likely that social and environmental factors contributed. For example, in Mayotte, CHIKV seroprevalence was higher in households with structural risk factors for mosquito breeding or exposure, such as open yards and outdoor toilets.[17] In two neighborhoods in Rio de Janeiro, the highest seroprevalence of dengue was seen in the busiest areas, where shops, schools, and transport hubs were located.[18]

The asymptomatic rate of 17.5% in our sample is within the range of 16.7–27.8% reported in other studies.[11,12,15,16] This rate is much lower than that of dengue, which has asymptomatic rates of over 70%.[18,19] In the present study, 33 of 38 (86.8%) symptomatic participants were seropositive, similar to the 53 of 63 (84.1%) symptomatic cases with laboratory-confirmed CHIKV infection reported during the outbreak.[2,3] Some of the suspected cases could be caused by other infections giving similar clinical symptoms, such as dengue. Symptom-based surveillance is, therefore, not enough to accurately determine the attack rate during CHIKV outbreaks, and asymptomatic people must also be tested. Asymptomatic cases would have played a role in community transmission, because they would not be confined to their homes like ill cases and would remain mobile and active during their viremic stage.

The acute symptoms reported in this study were similar to other outbreaks. Long-term sequelae, especially arthralgia, have been reported in uncontrolled studies.[20] However, the only study to date using matched controls found that CHIKV-infected patients had only slightly reduced physical scores for quality of life, with no impact on medical care received or mental scores.[21] In support of this, we found no residual physical dependence or depression in our sample. This supports the

TABLE 3
Laboratory-confirmed attack rates and symptomatic:asymptomatic case ratios reported in previous CHIKV outbreaks

Site (year of outbreak)	CHIKV genotype*	Estimated population size	Sample size tested for CHIKV (%)	Attack rate (%)	Asymptomatic rate (%)	Symptomatic: asymptomatic ratio	Reference
Bagan Panchor, Malaysia (2006)	Asian	1,500	72 (4.8)	55.6	17.5	4.7	This study
Castiglione di Cervia, Italy (2007)	CEA	2,000	325 (16.3)	10.2	17.9	4.6	15
Mayotte (2006)	CEA	175,000	1,154 (0.7)	37.2	27.8	2.6	11
Réunion Island (2005–2006)	CEA	787,836	2,442 (0.3)	38.2	16.7	5.0	12
Grande Comore, Comoros (2005)	CEA	340,000	331 (0.9)	63	–	–	13
Lamu Island, Kenya (2004)	CEA	18,000	288 (1.6)	75	–	–	14
Barangay Pulo, Philippines (1996)	Unknown (probably Asian)	500	298 (59.6)	65.5	23.3	3.3	16

* CEA = Central/East African.

need to better understand long-term outcomes of CHIKV infection.

There are limitations of our study. Over one-half of the participants were housewives and children < 18 years, those most likely to be at home during the daytime when the study was conducted. However, there were no significant differences in symptomatic disease or seropositivity among the different occupations. Because the study was carried out 1 year after the outbreak, there is the potential of recall bias. Finally, we cannot exclude the possibility that asymptomatic seropositive residents were actually infected before or after the outbreak. However, all the symptomatic residents reported symptoms only during the outbreak, and because CHIKV infection is mostly symptomatic, this suggests that the disease was active only at that time.

In summary, the outbreak of Asian CHIKV in Bagan Panchor was comparable with recent outbreaks of the epidemic CEA genotype in terms of acute symptoms, attack rate, and asymptomatic infection rate. The high attack rate and the duration of arthralgia for as long as 3 months show the impact of a CHIKV outbreak on a susceptible population. Seroprevalence was greater in the part of the village with more human activity, which suggests that busy communal areas should be specifically targeted with vector control measures during an outbreak.

Received May 17, 2010. Accepted for publication September 28, 2010.

Acknowledgments: We acknowledge the kind cooperation of the residents of Bagan Panchor during this study. The authors thank Nordin Mohamed, Norizan Moez, the Master of Public Health students, and the medical students who were part of the investigation team.

Financial support: This study was funded by the China Medical Board and University Malaya (High Impact Research Grant Allocation J-73565).

Authors' addresses: Said Mas Ayu and Noran Naqiah Hairi, Department of Social and Preventive Medicine, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia, E-mails: mas_ayu@um.edu.my and noran@um.edu.my. Li Rong Lai, Yoke Fun Chan, and I-Ching Sam, Tropical Infectious Diseases Research and Education Centre, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia, E-mails: amylaibeloved@hotmail.com, chanyf@um.edu.my, and jicsam@um.edu.my. Ahmad Hatim, Department of Psychological Medicine, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia, E-mail: hatim@um.edu.my. Asiah Ayob, Communicable Diseases Control Unit, State Health Department, Ipoh, Perak, Malaysia, E-mail: drasiahayob@moh.gov.my.

REFERENCES

1. Lam SK, Chua KB, Hooi PS, Rahimah MA, Kumari S, Tharmaratnam M, Chuah SK, Smith DW, Sampson IA, 2001. Chikungunya infection—an emerging disease in Malaysia. *Southeast Asian J Trop Med Public Health 32:* 447–451.
2. AbuBakar S, Sam IC, Wong PF, MatRahim N, Hooi PS, Roslan N, 2007. Reemergence of endemic Chikungunya, Malaysia. *Emerg Infect Dis 13:* 147–149.
3. Kumarasamy V, Prathapa S, Zuridah H, Chem YK, Norizah I, Chua KB, 2006. Re-emergence of Chikungunya virus in Malaysia. *Med J Malaysia 61:* 221–225.
4. Pavri K, 1986. Disappearance of Chikungunya virus from India and Southeast Asia. *Trans R Soc Trop Med Hyg 80:* 491.
5. Noridah O, Paranthaman V, Nayar SK, Masliza M, Ranjit K, Norizah I, Chem YK, Mustafa B, Kumarasamy V, Chua KB, 2007. Outbreak of Chikungunya due to virus of Central/East African genotype in Malaysia. *Med J Malaysia 62:* 323–328.
6. Sam IC, Chan YF, Chan SY, Loong SK, Chin HK, Hooi PS, Ganeswrie R, AbuBakar S, 2009. Chikungunya virus of Asian and Central/East African genotypes in Malaysia. *J Clin Virol 46:* 180–183.
7. Google Maps, 2010. *Bagan Panchor Dalam*. Available at: http://maps.google.com/maps?q=4.533+100.633+(Bagan+panchor+dalam)&ll=4.533,100.633&spn=05.0,05.0&t=k&hl=en. Accessed July 31, 2010.
8. Mahoney FI, Barthel DW, 1965. Functional evaluation: the Barthel index. *Md State Med J 14:* 61–65.
9. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC, 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry 59 (Suppl 20)*: 22–33.
10. Marchette NJ, Rudnick A, Garcia R, 1980. Alphaviruses in peninsular Malaysia. II. Serological evidence of human infection. *Southeast Asian J Trop Med Public Health 11:* 14–23.
11. Sissoko D, Moendandze A, Malvy D, Giry C, Ezzedine K, Solet JL, Pierre V, 2008. Seroprevalence and risk factors of Chikungunya virus infection in Mayotte, Indian Ocean, 2005–2006: a population-based survey. *PLoS One 3:* e3066.
12. Gérardin P, Guernier V, Perrau J, Fianu A, Le Roux K, Grivard P, Michault A, de Lamballerie X, Flahault A, Favier F, 2008. Estimating Chikungunya prevalence in La Réunion Island outbreak by serosurveys: two methods for two critical times of the epidemic. *BMC Infect Dis 8:* 99.
13. Sergon K, Yahaya AA, Brown J, Bedja SA, Mlindasse M, Agata N, Allaranger Y, Ball MD, Powers AM, Ofula V, Onyango C, Konongoi LS, Sang R, Njenga MK, Breiman RF, 2007. Seroprevalence of Chikungunya virus infection on Grande Comore Island, Union of the Comoros, 2005. *Am J Trop Med Hyg 76:* 1189–1193.
14. Sergon K, Njuguna C, Kalani R, Ofula V, Onyango C, Konongoi LS, Bedno S, Burke H, Dumilla AM, Konde J, Njgenga MK, Sang R, Breiman RF, 2008. Seroprevalence of Chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *Am J Trop Med Hyg 78:* 333–337.
15. Moro ML, Gagliotti C, Silvi G, Angelini R, Sambri V, Rezza G, Massimiliani E, Mattivi A, Grilli E, Finarelli AC, Spataro N, Pierro AM, Seyler T, Macini P, Chikungunya Study Group, 2010. Chikungunya virus in north-eastern Italy: a seroprevalence study. *Am J Trop Med Hyg 82:* 508–511.
16. Retuya TJA Jr, Ting DL, Dacula BD, Lanada JM, Roque VG Jr, Hugo CT, Ogayon JG, Abad-Viola GB, Roces MCR, 1998. Chikungunya fever outbreak in an agricultural village in Indang, Cavite, Philippines. *Philipp J Microbiol Infect Dis 27:* 93–96.
17. Raude J, Setbon M, 2009. The role of environmental and individual factors in the social epidemiology of Chikungunya disease on Mayotte Island. *Health Place 15:* 689–699.
18. Honório NA, Nogueira RMR, Codeço CT, Carvalho MS, Cruz OG, Magalhães MAFM, de Araújo JMG, de Araújo ESM, Gomes MQ, Pinheiro LS, da Silva Pinel C, Lourenço-de-Oliveira R, 2009. Spatial evaluation and modeling of dengue seroprevalence and vector density in Rio de Janeiro, Brazil. *PLoS Negl Trop Dis 3:* e545.
19. Porter KR, Beckett CG, Kosasih H, Tan RI, Alisjahbana B, Rudiman PIF, Widjaja S, Listiyaningsih E, Ma'Roef CN, McArdle JL, Parwati I, Sudjana P, Jusuf H, Yuwono D, Wuryadi S, 2005. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. *Am J Trop Med Hyg 72:* 60–66.
20. Borgherini G, Poubeau P, Jossaume A, Gouix A, Cotte L, Michault A, Arvin-Berod C, Paganin F, 2007. Persistent arthralgia associated with Chikungunya virus: a study of 88 adult patients on Reunion Island. *Clin Infect Dis 47:* 469–475.
21. Souahoro MK, Gérardin P, Boëlle PY, Perrau J, Fianu A, Pouchot J, Malvy D, Flahault A, Favier F, Hanslik T, 2009. Impact of Chikungunya virus infection on health status and quality of life: a retrospective cohort study. *PLoS One 4:* e7800.